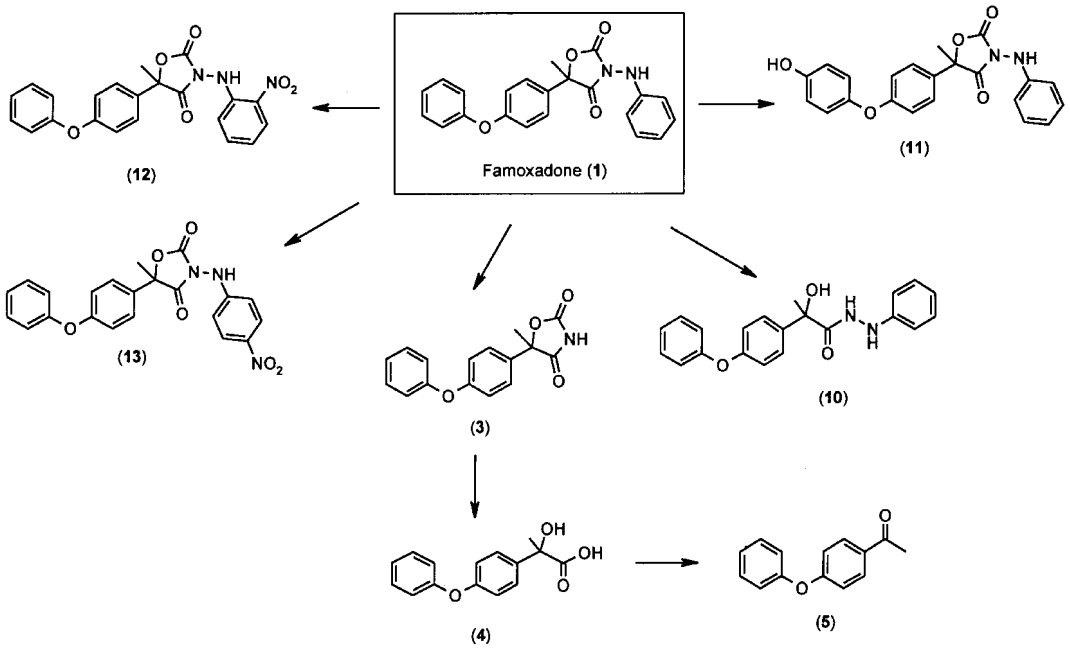

Figure 4. Pathway for primary degradation of famoxadone in soil.

2.2 Fate in the soil environment

Famoxadone degrades rapidly in the soil under laboratory aerobic and anaerobic incubation conditions (DT_{50} 6 and 28 days, respectively), mainly by both hydrolytic and microbial action. [^{14}C] carbon dioxide and unextractable bound residues are recovered as primary terminal residues. The DT_{50} of famoxadone in an aerobic aqueous sediment metabolism study is <1 day. The major degradation pathways include the hydroxylation of the parent molecule at the 4′-phenoxyphenyl position to yield compound **11** (Fig 4) and the hydrolytic cleavage of the oxazolidinedione-aminophenyl linkage to yield compounds **3, 4** and **5**. A novel nitration reaction (at the 2- or 4-phenylamino position) in soil yields the nitro-analogs of famoxadone (**12** and **13**). The soil degradation rate is accelerated when famoxadone is exposed to simulated sunlight (DT_{50} 12 *vs* 28 days). The soil adsorption coefficient of famoxadone (K_{oc}) is 3740. Aged soil column leaching studies show famoxadone and its soil metabolites to be compounds with low soil mobility potential. Significant movement and persistence of famoxadone in the soil environment is not anticipated. The primary soil degradation pathway of famoxadone is presented in Fig 4.

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Comparative metabolism of famoxadone in fish, plants and animals

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Abstract: The fate and comparative metabolism of famoxadone in fish, plants and animals were evaluated. Famoxadone residues were retained by the fish after exposure (BCF 2800), mainly in the viscera; however, rapid and complete elimination/depuration of the absorbed residues occurred within seven days after the exposed fish were placed in untreated water. Minimal absorption, translocation, and metabolism of famoxadone were observed in grape and potato plants after foliar treatment. Metabolism of famoxadone in the wheat plants, rats, goats, and poultry was extensive. Transfer of ^{14}C-residues to the wheat grain, milk, eggs, organs and tissues was minimal. Common metabolic reactions of famoxadone in plants and animals include aryl hydroxylation, cleavage of the anilino-oxazolidinedione and phenoxy-phenyl ether linkages, opening of the oxazolidinedione ring and conjugation.

Keywords: famoxadone; metabolism; bioconcentration; fish; plant; rat; goat; chicken

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(*Received 24 June 1998; accepted 5 January 1999*)

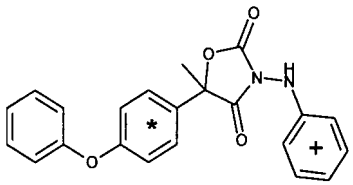

Figure 1. Structure of famoxadone. + Denotes [PA-^{14}C] famoxadone. *Denotes [POP-^{14}C] famoxadone.

Table 1. Physicochemical and aquatic toxicity data for famoxadone.

Solubility in unbuffered water (20 °C)		52 μg litre^{-1}
Aqueous stability (DT_{50}; 25 °C)	pH5	41 days
	pH7	2 days
	pH9	1.6 h
Vapour Pressure (20 °C)		6.4×10^{-4} Pa
Henry's Law Constant		4.6×10^{-3} Pa m^3 mole^{-1}
Log K_{ow} (pH7)		4.65
Acute LC_{50} (sunfish, dynamic)		11 μg litre^{-1}
Fish early life stage NOEC (trout)		1.4 μg litre^{-1}
Acute 48-h EC_{50} (Daphnia)		12 μg litre^{-1}

1 INTRODUCTION

Famoxadone [Famoxate® DPX-JE874, 3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione] is a novel fungicide being developed by DuPont Agricultural Products. Famoxadone is effective against a broad spectrum of fungal pathogens such as grape downy mildew, potato/tomato early and late blight, cucumber downy mildew, wheat leaf and glume blotch.[1] This summary describes the fate and comparative metabolism of famoxadone in fish, plants and animals. Studies were conducted using famoxadone labelled in the phenoxyphenyl and phenylamino rings, abbreviated as [*POP*-^{14}C]-and [*PA*-^{14}C] famoxadone respectively.

2 EXPERIMENTAL AND RESULTS

2.1 Bioconcentration potential in fish

Famoxadone has low water solubility and is toxic to various aquatic organisms. Key physicochemical properties and aquatic toxicity data are summarised in Table 1.

The bioaccumulation potential of [^{14}C] famoxadone in bluegill sunfish under a dynamic flow-through system at constant concentrations of 0.24 and 2.4 μg litre^{-1} (representing 2.5–25% of the dynamic LC_{50}) over a period of 28 days was examined. Rapid uptake of the radioactivity was observed, whole fish ^{14}C-residues reaching a plateau after seven to nine days. The majority (*c*75%) of the ^{14}C-residues were localised in the viscera at the end of the uptake phase. The steady-state bioconcentration factor (BCF) for [^{14}C]famoxadone in whole fish was approximately 2800 at both test concentrations. Depuration of ^{14}C-residues was rapid following the transfer of the exposed fish to untreated, flowing water, >95% of the residues in the whole fish, fillet, and viscera being eliminated by the end of the 14-day depuration period. Famoxadone was the primary radioactive component recovered from the fish tissues. Although famoxadone exhibits a high BCF potential, it is rapidly degraded in the aquatic environment and is extensively eliminated from exposed fish, so that it is concluded that it will have minimal impact to the aquatic environment.

2.2 Metabolism in plants

The metabolic fate of famoxadone was evaluated in three different crop species: grapes (representative of fruits and fruiting vegetables, including tomatoes), wheat (representative of cereals), and potatoes (representative of vegetables and root crops). Testing parameters such as formulation types of the test substance, number of applications, application rate and timing, and sample collection intervals were consistent with the intended use pattern and the Good Agricultural Practices for famoxadone.

2.2.1 Grapes

Minimal systemic translocation and metabolism of famoxadone were observed in and on both grape foliage and berries after direct foliar applications. The majority of the ^{14}C-residues were characterised as surface residues which could be recovered by simple surface washing with aqueous acetonitrile. More than 90% of the recovered ^{14}C-residues were identified as undegraded famoxadone. 1-(4-Phenoxyphenyl)ethanone (**2**; see Fig 2), a cleavage product of famoxadone, was observed as the only degradation product (<5%).

2.2.2 Potatoes

When famoxadone was applied directly onto potato foliage; the tubers subsequently contained <0.01 mg kg^{-1} [^{14}C]famoxadone-equivalent residues, indicating a lack of translocation. Minimal metabolism of famoxadone was observed in and on the potato foliage: the majority of the ^{14}C-residues were characterised as surface residues which were recovered by simple surface washing with aqueous acetone. Of the recovered ^{14}C-residues, >80% was present as undegraded famoxadone. Cleavage of famoxadone to yield compound **2** was observed as the only degradation process (<5%)

2.2.3 Wheat

When famoxadone was applied directly onto wheat foliage, negligible translocation of ^{14}C-residues to the wheat grain, which contained 0.01–0.02 mg kg^{-1} [^{14}C]famoxadone-equivalent residues, was observed. The majority of the ^{14}C-residues were foliage surface residues which were recovered by simple solvent washings with aqueous acetonitrile. In addition to the undegraded famoxadone, hydroxylation products **3** and **4**, and a conjugation product, **5** (Fig 2) were identified as primary metabolites. The hydrolysis cleavage products **6, 7, 8** and **9** were observed as

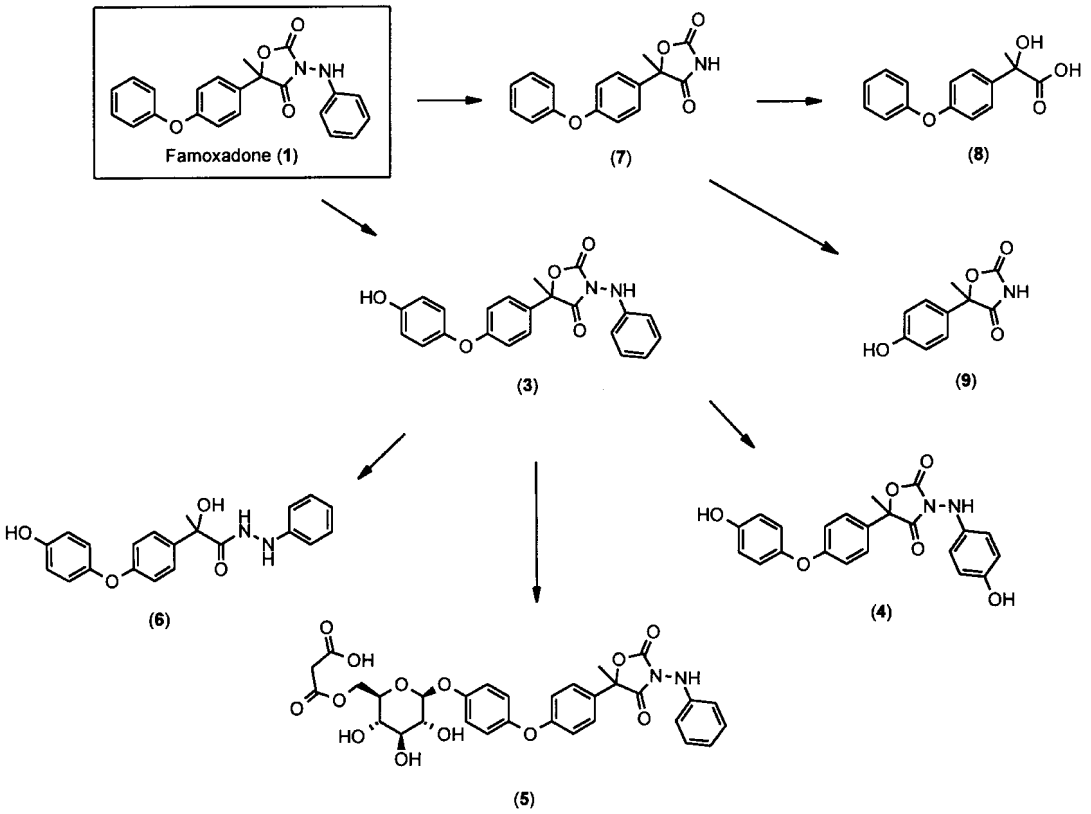

Figure 2. Preposed metabolic pathway of famoxadone in plants.

minor metabolites. Except for famoxadone, no individual metabolite or degradation product accounted for >10% of the total plant ^{14}C-residues. Significant binding or incorporation of the ^{14}C-residues to the plant materials (characterised as unextractable bound residues) was observed. The proposed metabolic pathway of famoxadone in and on wheat plants is presented in Fig 2.

2.3 Metabolic fate in animals

The metabolism of famoxadone was studied in laboratory rats, goats and poultry. Laboratory rats were administered a single oral dose of [^{14}C]famoxadone at 50 or 250 mg kg^{-1} body weight. Lactating goats and egg-laying hens received orally administered [^{14}C]famoxadone at dose levels equivalent to, or greatly exceeding, the anticipated maximum daily dietary intake of famoxadone residues from the various animal feed items. The dosing regimen for the goats and hens was 5 mg kg^{-1} diet twice daily for three days, and 10 mg kg^{-1} diet, once daily for seven days, respectively. Egg and milk residues were evaluated daily. Animals were killed within 24 h of the last dosing, and tissue residues were determined.

2.3.1 Rat

A rapid elimination of the administered radioactive dose in the urine and faeces of normal and bile-cannulated rats was observed. Faecal excretion was the primary elimination route, >75% of the administered radioactive dose being eliminated in this way within the initial 24 h post-dosing. In general, approximately 10 and 90% of the administered radioactive doses were recovered in the urine and faeces, respectively. In the non-cannulated test animals, three radioactive components were observed in the faecal extracts, unmetabolised [^{14}C]famoxadone being the major component. Aryl hydroxylation was the major metabolic reaction, yielding the mono- and dihydroxylated parent molecules (compounds **3** and **4,** respectively) as principal faecal metabolites. Opening of the parent oxazolidinedione-ring yielded **10** (Fig 3) as a minor metabolic product. In the urinary excreta from [*POP*-^{14}C]famoxadone-treated non-cannulated animals, only one major radioactive component was observed. Cleavage of the anilino-oxazolidinedione linkage of hydroxylated famoxadone (compound **3** and/or **4**) followed by sulfate conjugation of the aryl hydroxy-moiety yielded **11** as the major urinary product. In the urinary excreta from [*PA*-^{14}C]famoxadone-treated animals, the *O*-acetylated hydroxyaniline, **12**, was the principal product detected.

A larger portion (up to 40%), of the administered dose was rapidly excreted in the bile of cannulated than of non-cannulated rats. Again, only a small amount of radioactive dose (up to 6%) was recovered in the urine, faecal excretion being the major elimination pathway. Unmetabolised famoxadone was the only radiolabeled component detected in the faecal extract, but was not detected in the bile samples. Several polar conjugated products were observed in the bile samples, after treatment with β-glucuronidase/sulfatase enzymes, the released metabolites being qualitatively identified as compounds **3, 4, 6, 13** and

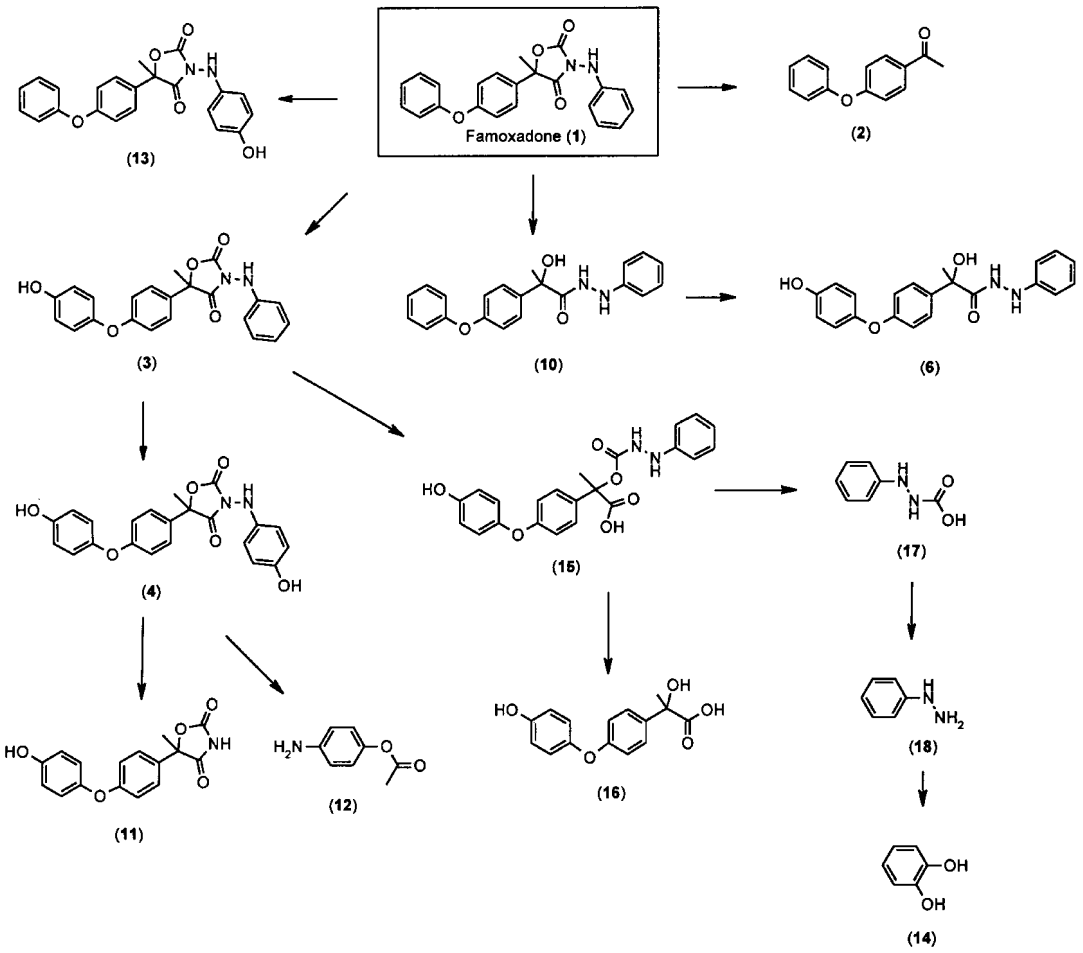

Figure 3. Primary metabolic pathway of famoxadone in cannulated and non-cannulated rats.

catechol (**14**) from the bile of [*PA*-^{14}C]famoxadone-treated animals and compounds **3, 4, 11, 13, 15**, and **16** from the bile of [*POP*-^{14}C]famoxadone-treated animals. The proposed mechanism of the formation of catechol (**14**) is *via* the oxazolidindione ring opening (to yield **15**) followed by cleavage of the ester linkage to yield **17** and phenylhydrazine (**18**). The primary metabolic pathway of famoxadone in non-cannulated and cannulated laboratory rats is consistent and is presented in Fig 3.

2.3.2 Goats

Rapid elimination of the administered radioactive dose was observed in the faeces and rumen contents (*c*85%). Urine was a minor elimination route (*c*3%). Absorption was minimal, since the transfer of [^{14}C]famoxadone-equivalent residues to the milk, muscle, and kidney tissues was negligible (<0.01–0.03 mg kg^{-1}). ^{14}C-Residues in the fat and liver tissues were low, ranging from 0.07 to 0.17 mg kg^{-1}. The combined milk and tissue ^{14}C-residues accounted for <0.6% of the total administered dose.

Unmetabolised famoxadone was the major component detected in the faeces, milk, and fat tissues. Hydroxylation (to yield compounds **3** and **4**), hydrolysis (to yield compound **6**), and several hydrolysis cleavage products (compounds **11, 19, 20, 21**, etc) were detected in the faeces, urine and liver tissue extracts. Aniline (**20**) and hydroxyaniline (**21**) underwent further *N*-acetylation to yield compounds **22** and **23**, respectively. Stereoselective metabolism of famoxadone enantiomers in the goat was not evident. The metabolic pathway of famoxadone in the lactating goat is presented in Fig 4.

2.3.3 Chicken

Rapid elimination of the administered radioactive dose in the chicken excreta (*c* 90%) was observed. Absorption was minimal, since the transfer of ^{14}C-residues to the egg white, fat, muscle, and skin tissues was negligible (<0.01–0.02 mg kg^{-1}). Low levels of ^{14}C-residues were detected in the egg yolk (<0.01–0.07 mg kg^{-1}) and liver (0.06–0.3 mg kg^{-1}). The combined egg and tissue ^{14}C-residues accounted for <0.2% of the total administered dose.

A complex metabolic profile was observed in the chicken excreta. In addition to undegraded famoxadone as the major component, approximately 15 metabolites were identified. These metabolites were generated from multiple metabolic reactions which included aryl hydroxylation, opening of the oxazolidinedione ring and the cleavage of the anilino-oxazolidinedione and the phenoxy-phenyl ether linkages. Stereoselective metabolism of famoxadone enantiomers in the egg-laying hens was not evident. Famoxadone was the primary component in the egg yolk.

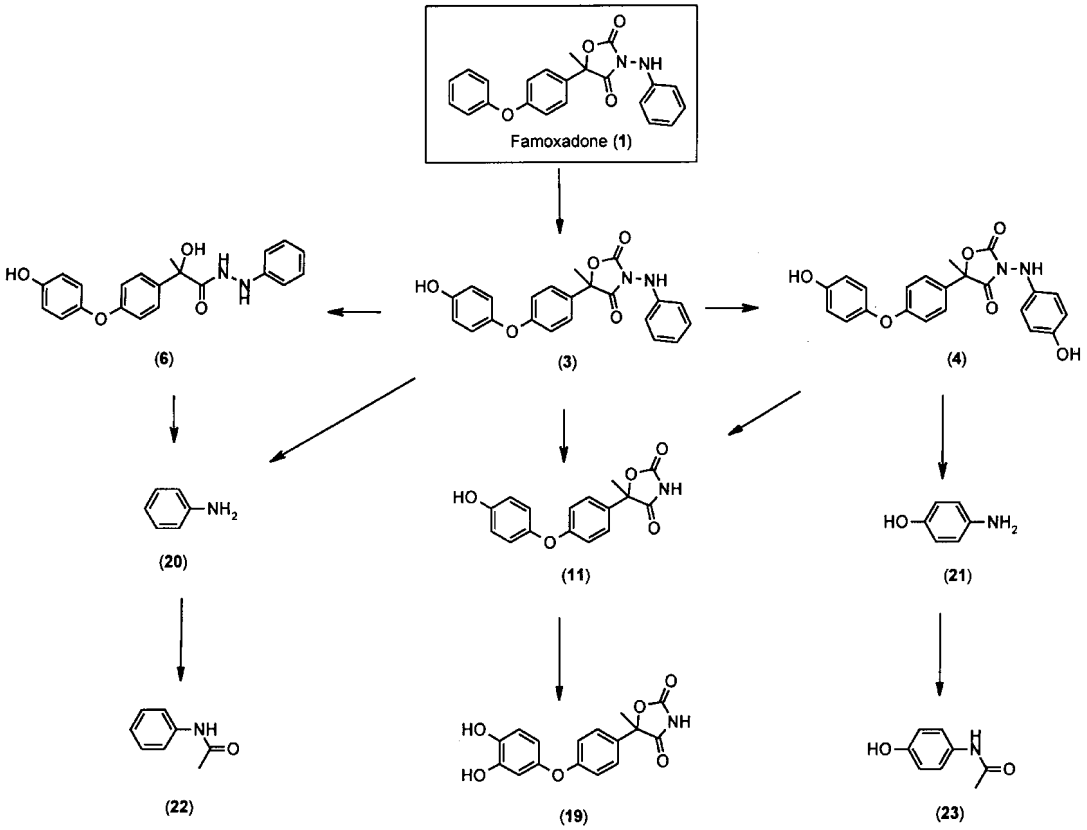

Figure 4. Famoxadone metabolic pathway in goats.

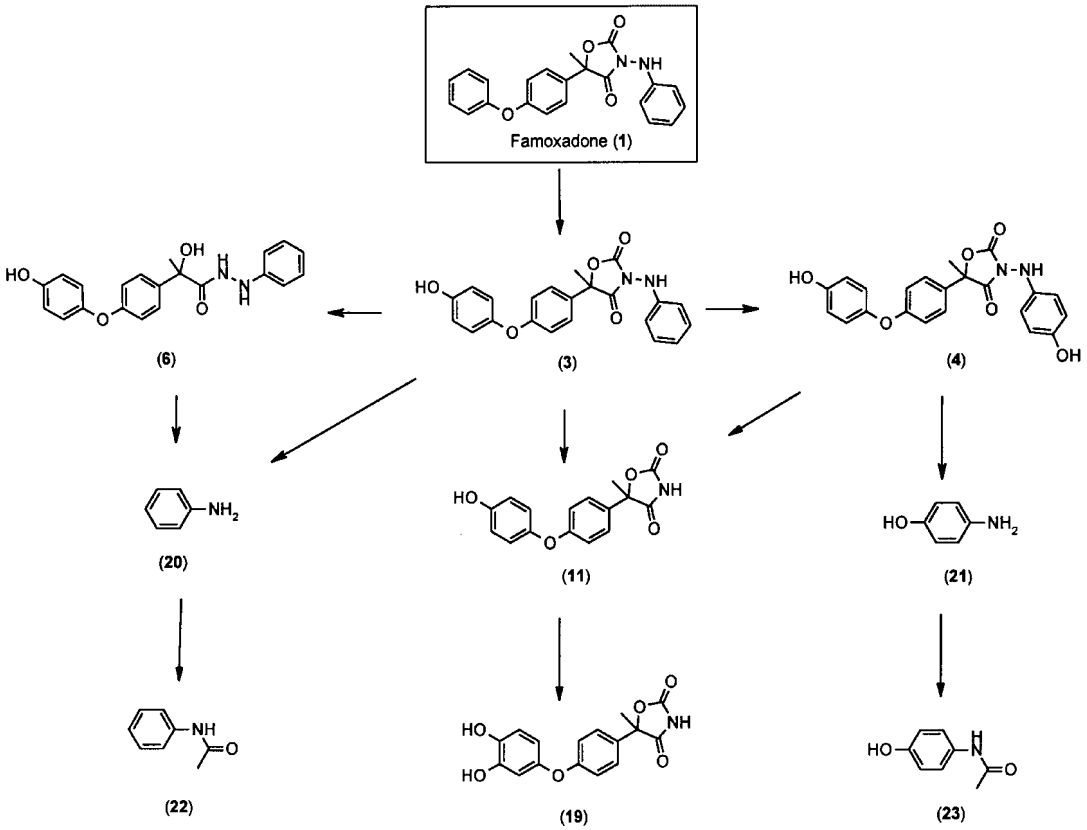

Figure 5. Metabolic pathway of famoxadone in chicken.

Hydroxylation products (compounds **3** and **4**) were major components in the liver extract. The metabolic pathway of famoxadone in chicken is presented in Fig 5.

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The sex-specific sulfation of the major metabolite of the novel fungicide cyprodinil in the rat

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Abstract: The metabolism of cyprodinil, a novel broad-spectrum fungicide, was investigated in rats. After single oral administration of 0.5 or 100 mg kg^{-1} body weight, [*phenyl-U*-^{14}C]cyprodinil was rapidly eliminated, principally in the urine. The metabolite pattern in urine exhibited a significant sex-related difference with respect to the major metabolite. Males and females both produced a dihydroxy metabolite, *N*-4-(hydroxyphenyl)-4-cyclopropyl-5-hydroxy-6-methylpyrimidin-2-ylamine. Female rats conjugated this metabolite with sulfate exclusively at the 5-hydroxypyrimidinyl moiety, while males formed equal amounts of the monosulfate and a disulfate conjugate. The sex dimorphism in the conjugation reaction indicates the involvement of a sex-specific sulfotransferase that catalyzed the transfer of the second sulfate group.

Keywords: cyprodinil; fungicide; metabolism; conjugation; sulfotransferase; sulfation; disulfate; sex dimorphism

1 INTRODUCTION

Cyprodinil, (4-cyclopropyl-6-methyl-*N*-phenylpyrimidin-2-ylamine), belongs to the novel class of pyrimidinamine fungicides. Cyprodinil exhibits a broad-spectrum activity against a variety of phytopathogenic fungi, making it suitable for protection of cereals, grapes, apples and vegetables.[1] The pyrimidinamines interfere with methionine biosynthesis of phytopathogenic fungi, a pathway specific to microorganisms and plants.[2] As part of the toxicological evaluation of cyprodinil, the urinary metabolites were identified after oral administration to rats.

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2 EXPERIMENTAL

[*Phenyl-U*-^{14}C] cyprodinil was administered by gastric intubation to two groups of young rats (Tif: RAI SPF, 195–215 g body weight), each consisting of five males and five females. One group of rats received a single low dose of 0.5 mg kg^{-1} body weight, while the rats of the second group received the high dose of 100 mg kg^{-1}. The test substance was dissolved in ethanol+polyethylene glycol 200+ water (1+2+1 by volume) and administered at *c*4 ml kg^{-1}. The rats were housed in metabolism cages throughout the study and permitted free access to food and water. The radioactivity in urine was determined by liquid scintillation counting. Faeces samples were homogenized manually with a pestle after addition of water (*c*1 ml), and aliquots were combusted in a Tri-Carb Sample Oxidizer (Packard). The 0–48 h urine was analysed by HPLC on a Zorbax ODS C18 column (4.6 mm ID × 250 mm) using a Beckman chromatography system connected to a Ramona A radioactivity flow monitor (Raytest). The solvent system was 10 mM ammonium formate + methanol (100+0 by volume) for 5 min then to 50+50 by volume over 40 min, to 10+90 by volume over 15 min, then held at this composition; the flow rate was 1 ml min^{-1}. The quantitative distribution of metabolites was determined by integration of the radioactivity detector signal using the Nelson Analytical Turbochrom® software. Metabolites present in urine were isolated by HPLC, applying successive chromatography on reverse-phase columns. Their structures were elucidated by [^{1}H]NMR and Fast Atom Bombardment mass spectroscopy.

3 RESULTS

The administered radioactivity was rapidly eliminated irrespective of the dose level and the sex of the animals. Within 48 h 92–97% of the dose had been excreted (Table 1). The principal route of elimination was the urine, since 53–60% of the dose was excreted in this over 168 h. Lower amounts were eliminated with the faeces (37–45%). Within seven days the administered radioactivity had been almost completely eliminated.

HPLC analysis of male rat urine revealed a metabolite pattern that was dominated by two metabolites, designated M1 and M2 (Fig 1). In contrast to that of males, the urine of female rats contained metabolite M1 but not M2. The distribution of both metabolites was independent of the dose level (Table 2). The combined metabolites M1 and M2 amounted to 30% of the dose in male rats, which was in the same range as the percentage of metabolite M1 alone in females (31–35%). The distribution of all other urinary metabolites (M3, M4, M5, M6 and M7) did not show a sex- or dose-related difference.

Cyprodinil is metabolized by sequential oxidation of the phenyl and pyrimidinyl rings (Fig 2). Hydroxylation of the phenyl or the pyrimidinyl ring yields the 4-hydroxyphenyl or 5-hydroxypyrimidinyl metabolites